Induced Helicity in Short Peptides

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Certain short amino acid chains preferentially form alpha helical segments. Interestingly, the introduction of non-native end groups can induce helical structure beyond that observed for unmodified peptides of identical sequence, although the chemical mechanism for this effect is not yet known. Our collaborators have synthesized two series of short peptides: one with all native residues, and another with non-native N-terminus groups. The goal of our research is to model these peptides using molecular dynamics in an attempt to 1) Determine a reliable metric which can be used to quantify the percent helicity of a given peptide, and 2) To analyze the structural and dynamical characteristics of non-native N-terminus caps which increase the helical content of short peptides. The analysis of the native peptides is nearly complete, and preliminary results indicate that a simple measure of helicity can be obtained from the phi, psi dihedrals which reas onably reproduce experimental helicities obtained from CD spectra.

Introduction:

Synthesizing peptides which fold into specific secondary structures is desirable but technically challenging. Such peptides are useful in protein structure- function studies, and have direct applicability to peptide based drugs. Our collaborators have examined the α-helical content of unmodified short peptides as a function of the amino and carboxyl terminating amino acids to determine which residues support the greatest helical content (Forood, Feliciano, and Nambiar; *Proc. Natl. Acad. Sci. USA,* vol. 90, 1993). By modifying the N-terminus with sulfur containing moieties of increasing oxidation our collaborators have shown that the helical content increases with the sulfur oxidation state and hydrogen bonding capacity(Forood, Feliciano, and Nambiar; *J. Am. Chem. Soc. 1994, 116, 6935-6936*).

This research is aimed at elucidating the chemical interactions which stabilize and initiate helical structure via molecular dynamics simulations. Pauling's model of the α-helix (Pauling, Corey, and Branson; *Proc. Natl. Acad. Sci. USA, vol 37, 1951*) consists of repeating hydrogen bonds between the backbone carbonyl and amide groups four residues away. This H-bonding scheme leaves the first four NH and last four CO groups unpaired. It is believed that residues with side chains capable of H-bonding to these groups stabilizes the helix. Similarly, capping groups that facilitate more H-bonds with the peptide backbone should show an increase in helicity.

Our long term goal is to answer several key questions about this phenomenon. What are the interactions stabilizing α-helices? In particular, what is the role of end groups on stability? Can a reproducible measure of helicity be obtained from MD Simulations?

		% Helicity (CD)[1]
	Unmodified Peptides	
1	Asp-Pro-Ala-Glu-Ala-Ala-Lys-Ala-Ala-Ala-Gly-Arg-amide	25.1
2	Ala-Pro-Ala-Glu-Ala-Ala-Lys-Ala-Ala-Ala-Gly-Arg-amide	2.4
3	Asn-Pro-Ala-Glu-Ala-Ala-Lys-Ala-Ala-Ala-Gly-Arg-amide	24.4
4	Gln-Pro-Ala-Glu-Ala-Ala-Lys-Ala-Ala-Ala-Gly-Arg-amide	4.8
	Modified Peptides	
	R-Ala-$(Ala)_3$-Glu-$(Ala)_3$-Lys-$(Ala)_4$-Tyr-Arg-amide	
R =	H	17.4
	CH_3-S-CH_2CH_2CO-	32.1
	CH_3-S(O)-CH_2CH_2CO-	39.9
	$CH_3S(O)_2$-CH_2CH_2CO-	62.8

Table 1: Experimental results for the unmodified 12 residue peptides of interest (simulation results are given for 1 and 2 only), and the 15 residue template with the various N-Caps.

Results:

Preliminary results for peptides 1 and 2 indicate that it is possible to distinguish the percent helicities of each polymer. Peptide 1 has an average helicity of 47.3 ± 9.8%, and 32.8 ± 10.6% for peptide 2 calculated from the φ, ψ dihedrals. The average difference in helicities is 14.7% over a 1.3 ns interval.The percent helicities as a function of time are given in figure 1. Figure 2 provides a comparison of peptide 1 and 2 helicities calculated from dihedrals and H-bonds.

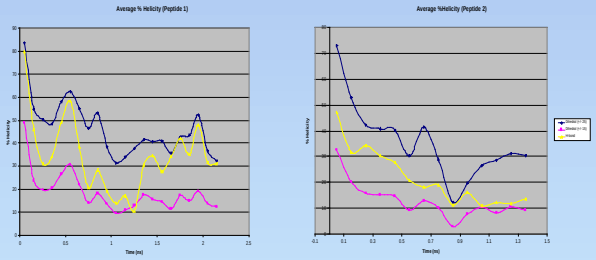

Figure 1: % helicity over time calculated from dihedrals and H-bonds (see Methods)

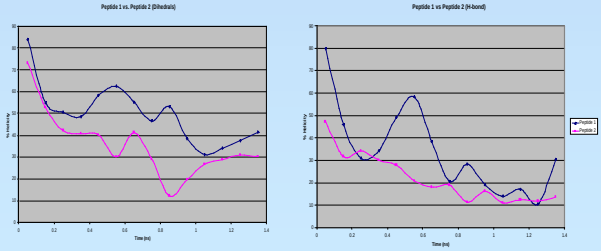

Figure 2: Comparison of helical content for peptides 1 and 2 using dihedrals and H-bonds.

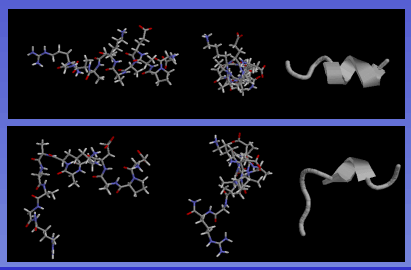

Figure 3: Above peptide 1 at 0.50072 ns, and below at 1.0016 ns depicted from left to right as side, end on, and cartoon views. The helicities are 80% and 10% respectively based on dihedral measurements.

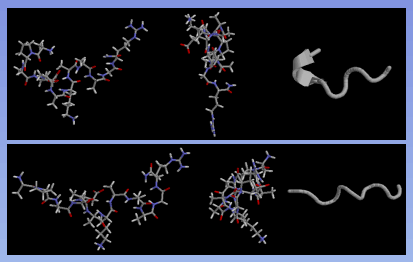

Figure 4: Above peptide 2 at 0.6014 ns, and below at 0.8006 ns. Helicities are 70% and 0.0% respectively. Cartoon secondary structure not based on our α-helix criteria.

Discussion:

The results indicate a discernable difference in helicities for the two proteins studied. Although, the percent helicities do not closely match the values obtained from CD spectra the differences in helicities from the CD measurements and the simulations are similar. The fluctuations in the helical content of each polymer is large, and more simulation time is required to see if equilibrium is reached. Analysis of peptides 3 and 4 is also underway, and should yield similar results.

For the purposes of this study it appears that the percent helicity is best measured using the φ, ψ dihedrals. Secondary structure is often assigned based on H-bonding patterns, such as with the DSSP program (Kabsch and Sander; *Biopolymers*, vol. 22, 2577-2637 (1983)). Helical structural elements can be present when specific H-bonds are absent. The CD signal is a function of the periodic spiral of backbone carbonyls, and is unaffected by the presence of H-bonds.

These results warrant further investigation of the more complex substituted proteins listed in table 1. Having determined a reasonable measure of helicity we can now examine the substituent interactions with the backbone and determine if the effect increases or detracts from the helical content.

Methods:

The simulations were carried out using the Amber 7 suite of programs. Each peptide was constructed in a fully helical configuration with explicit solvent. 1591 waters were included with peptide 1 and 1638 included with 2. The MD simulations were carried out using SANDER at a constant temperature of 300K, and a constant pressure of 1 atm, after the waters were minimized and 15 ps of equilibration to reach the proper temperature and density. The trajectories were collected every 0.5 ps, and analyzed using the CARNAL module.

To analyze the helicities both the φ, ψ dihedrals and H-bonding patterns were inspected. Each amino acid was assigned to an helical conformation if the dihedrals were within $-89° < \varphi < -39°$ and $-66° < \psi < -16°$ (Hovmoller, Ohlson, and Zhou; *Acta. Cryst. (2002). D* 58, 768-776), which corresponds to ± 25° from ideal. A more rigid criteria was also applied for comparison (± 15°, see Fig. 1). A percent was then calculated by dividing by the maximum number of residues with the correct dihedrals(for 12 residues the max is 10 since the ends do not have both dihedrals defined). Similarly H-bonds were assigned based on the CO NH distances for the I, I+4 residue pairs. Again dividing by the maximum number of possible bonds (8 for 12 residue peptide) results in a percent helicity.

This work was performed under the auspices of the U.S. Department of Energy by University of California Lawrence Livermore National Laboratory under contract No. W-7405-Eng-48.

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